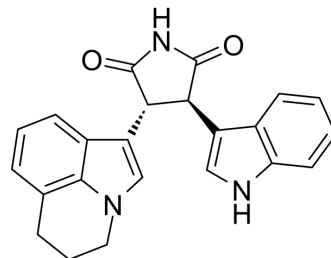


## Tivantinib

|                           |   |       |         |
|---------------------------|---|-------|---------|
| <b>Cat. No.:</b>          | HY-50686  |       |         |
| <b>CAS No.:</b>           | 905854-02-6   |       |         |
| <b>Molecular Formula:</b> | C <sub>23</sub> H <sub>19</sub> N <sub>3</sub> O <sub>2</sub> |       |         |
| <b>Molecular Weight:</b>  | 369.42  |       |         |
| <b>Target:</b>            | c-Met/HGFR; Apoptosis   |       |         |
| <b>Pathway:</b>           | Protein Tyrosine Kinase/RTK; Apoptosis                        |       |         |
| <b>Storage:</b>           | Powder  | -20°C | 3 years |
|                           |   | 4°C   | 2 years |
|                           | In solvent  | -80°C | 2 years |
|                           |   | -20°C | 1 year  |



### SOLVENT & SOLUBILITY

|   |  |                          |              |            |            |
|---|--|--------------------------|--------------|------------|------------|
| <b>In Vitro</b>   | DMSO : 100 mg/mL (270.69 mM; Need ultrasonic)  |                          |              |            |            |
|   |  | Solvent<br>Concentration | Mass<br>1 mg | 5 mg       | 10 mg      |
|   | <b>Preparing Stock Solutions</b>   | 1 mM                     | 2.7069 mL    | 13.5347 mL | 27.0695 mL |
|   |  | 5 mM                     | 0.5414 mL    | 2.7069 mL  | 5.4139 mL  |
| 10 mM   |  | 0.2707 mL                | 1.3535 mL    | 2.7069 mL  |            |
| Please refer to the solubility information to select the appropriate solvent. |  |                          |              |            |            |
| <b>In Vivo</b>  | 1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline<br>Solubility: ≥ 2.5 mg/mL (6.77 mM); Clear solution |                          |              |            |            |

### BIOLOGICAL ACTIVITY

|                                     |  |
|-------------------------------------|--|
| <b>Description</b>                  | Tivantinib is a highly selective c-Met tyrosine kinase inhibitor with a K <sub>i</sub> of 355 nM.  |
| <b>IC<sub>50</sub> &amp; Target</b> | Ki: 355 nM (c-Met) <sup>[1]</sup>  |
| <b>In Vitro</b>                     | Tivantinib (ARQ 197) selectively inhibits c-Met activity in cell-free and cell-based assays. c-Met-expressing cancer cell lines treated with Tivantinib display either a dose-dependent loss of proliferative capacity or caspase-dependent apoptosis that positively correlates with either ligand-dependent c-Met activity or constitutively active c-Met. To examine the biochemical mode of inhibition of Tivantinib, kinetic analyses are done using recombinant human c-Met in a filtermat-based assay. The K <sub>m</sub> of ATP is 50.5±2.2 μM, which is similar to the K <sub>m</sub> value of ATP. In these kinetic studies, Tivantinib inhibits human recombinant c-Met with a calculated inhibitory constant (K <sub>i</sub> ) of ~355 nM. In vitro exposure to Tivantinib inhibits constitutive c-Met phosphorylation in HT29 and MKN-45 cells, and HGF-induced c-Met phosphorylation in MDA-MB-231 and NCI-H441 cells with an IC <sub>50</sub> of 100 to 300 nM <sup>[1]</sup> . Tivantinib is a low-molecular-weight compound, and is the first in class orally available |

selective inhibitor of c-Met<sup>[2]</sup>.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

#### In Vivo

Pharmacodynamically, the phosphorylation of c-Met in human colon xenograft tumors (HT29) is strongly inhibited by Tivantinib (ARQ 197), as assessed by a dramatic reduction of c-Met autophosphorylation 24 hours after a single oral dose of 200 mg/kg of Tivantinib. This same dosage in mice shows that tumor xenografts are exposed to sustained plasma levels of Tivantinib, consistent with the observed pharmacodynamic inhibition of c-Met phosphorylation and inhibition of proliferation of c-Met harboring cancer cell lines. A  $C_{max}$  of 5.73  $\mu\text{g/mL}$  (13  $\mu\text{M}$ ), an area under the concentration-time curve of 12.1  $\mu\text{g/mL h}$ , and a  $t_{1/2}$  of 2.4 hours are measured. Plasma levels of Tivantinib 10 hours after dosing are determined to be 1.3  $\mu\text{M}$ , >3-fold above the biochemical inhibitory constant of Tivantinib for c-Met<sup>[1]</sup>.

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## PROTOCOL

#### Kinase Assay <sup>[1]</sup>

Recombinant c-Met protein (50 ng) comprising residues 974 to 1390 is incubated in a reaction buffer [50 mM Tris-HCl (pH 7.5), 2 mM DTT, 0.1 mM  $\text{Na}_3\text{VO}_4$ , 10 mM  $\text{MgCl}_2$ , 1 mM EGTA, 0.02 mg/mL bovine serum albumin, and 10% glycerol] with various concentrations of Tivantinib or DMSO in a total volume of 20  $\mu\text{L}$ . After incubating for 20 minutes at room temperature, 20  $\mu\text{L}$  of 20  $\mu\text{M}$  poly-Glu-Tyr substrate and 20  $\mu\text{L}$  of increasing amounts of cold ATP containing 1.5  $\mu\text{Ci}$  of [ $\gamma$ -<sup>33</sup>P]ATP are added to initiate the reaction. The reaction is stopped after 5, 10, 20, 40, and 60 minutes by the addition of 10% phosphoric acid and 10  $\mu\text{L}$  aliquots of the reaction mixture are spotted onto P30 filtermat in triplicate. The filters are washed thrice for 5 minutes with 0.75% phosphoric acid and once with methanol for 2 minutes. The level of radioactivity is determined by using a Wallac TriLux MicroBeta liquid scintillation counter. Nonspecific binding is determined by conducting the assay in the absence of enzyme and then subtracting the value from each of the experimental values. Reaction rates are determined in the linear range of each reaction and GraphPad Prism software is used to calculate the  $K_m$  and  $V_{max}$  values<sup>[1]</sup>. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

#### Cell Assay <sup>[1]</sup>

HT29, MKN-45, MDA-MB-231, and NCI-H441 (lung cancer) human cancer cells are seeded in 96-well plates overnight in a medium with 10% FBS. Each cell line is optimized for seeding cell number to ensure a similar degree of confluence at the end of the experiment in nontreated (control) wells. The next day, cells are treated with different concentrations of Tivantinib for 24 hours at 37°C. After ARQ 197 treatment, the drug-containing medium is removed, and cells are washed twice with PBS and incubated in a drug-free medium for an additional 48 hours. Cells are then incubated and stained for 4 hours with the MTS reagent (final concentration of 0.5 mg/mL) per well and are lysed. The results are quantitated by spectrophotometry at  $\lambda=450\text{ nm}$ <sup>[1]</sup>.

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#### Animal Administration <sup>[1][2]</sup>

Mice<sup>[1]</sup>

Female athymic nude mice are acclimated to the animal housing facility for at least 1 week before the study. Efficacy studies are done in athymic mice bearing HT29, MKN-45, or MDA-MB-231 tumor xenografts to determine the effect of ARQ 197 on tumor growth. Tumor cells [ $5 \times 10^6$  (HT29) and  $8 \times 10^6$  (MKN-45 and MDA-MB-231) cells/animal] are inoculated s.c. on day 0. Tumor dimensions are measured by a digital caliper and tumor volumes are calculated as  $\text{length} \times \text{width}^2 / 2$ . When tumors reached a volume of  $\sim 100\text{ mm}^3$ , mice are randomized into groups and treated daily with orally administered vehicle control or 200 mg/kg Tivantinib formulated in polyethylene glycol 400/20% Vitamin E tocopheryl polyethylene glycol succinate (60:40) at 30 mg/mL, for 5 consecutive days, followed by a 2-day dosing holiday for four cycles. Therefore, each animal received a total of 20 doses. Results are expressed as mean tumor volume  $\pm$  SEM. To assess differences in tumor size between groups, a Mann-Whitney nonparametric t test is performed and significance is defined as  $P < 0.05$ .

Rats<sup>[2]</sup>

Six male Sprague-Dawley rats (180-220 g) are used to study the pharmacokinetics of Tivantinib. Diet is prohibited for 12 h before the experiment but water is freely available. Blood samples (0.3 mL) are collected from the tail vein into heparinized 1.5 mL polythene tubes at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after oral administration of Tivantinib (10 mg/kg). The samples are immediately centrifuged at 4000g for 8 min. The plasma obtained (100  $\mu\text{L}$ ) is stored at  $-20^\circ\text{C}$  until analysis. Plasma Tivantinib concentration versus time data for each rat is analyzed by DAS (Drug and statistics) software.

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## CUSTOMER VALIDATION

- Sci Transl Med. 2018 Jul 18;10(450):eaaq1093.
- J Pharm Anal. 2021 Jun 19.
- FEBS J. 2016 Jan;283(1):102-11.
- Cancers (Basel). 2022 Mar 19;14(6):1575.
- Cancer Res Treat. 2020 Jul;52(3):973-986.

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## REFERENCES

- [1]. Munshi N, et al. ARQ 197, a novel and selective inhibitor of the human c-Met receptor tyrosine kinase with antitumor activity. Mol Cancer Ther. 2010 Jun;9(6):1544-53.
- [2]. Bai YL, et al. Quantitative analysis of tivantinib in rat plasma using ultra performance liquid chromatography with tandem mass spectrometry. J Pharm Biomed Anal. 2016 Jul 15;126:98-102.
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